MODULE 4 SPECIAL STUDIES

4.1

SEMI-PERMEABLE MEMBRANE DEVICES

SEMI-PERMEABLE MEMBRANE DEVICES (SPMDS)

SPMDs

Semipermeable membrane devices (SPMDs) are integrative sampling devices which combine membrane diffusion and liquid-liquid partitioning to concentrate low to moderate molecular mass hydrophobic compounds from water (Huckins et al, 1996). SPMDs have some features that give them some advantages over monitoring contaminants in fish. SPMDs can be deployed in water to accumulate single, pulsed, or continuous contaminant releases over time. SPMDs are anchored to sample at specific locations, thereby avoiding any question of origin of contaminants caused by fish movement. SPMDs do not change function under stress, unlike gills of fish. There are no biotransformations or elimination like that in fish. There are, however, a number of conditions, such as temperature, DOC, solids which can effect the efficiency of these devices. And accumulation of contaminants does not occur by the same process of uptake in fish, thereby potentially limiting their use to accumulation in a relative sense.

Made of low density polyethylene lay-flat tubing (2.5 cm wide by 91.4 cm long), containing a thin film of neutral triolein and placed inside stainless steel canisters, SPMDs are deployed in the waterbody where they accumulate contaminants until retrieved. Laboratory handling of the SPMDs after field deployment involves the removal of biofouling, which is exterior debris and periphyton, before extraction. After this initial cleanup, the devices are then spiked with a cocktail of surrogates consisting of C-13 labeled analogs of the toxic native dioxin congeners in order to monitor recovery. After surrogate addition, individual SPMDs are dialyzed and the collected dialysates are cleaned by gel permeation chromatography followed by Florisil solid phase extraction. The extracts from the three SPMDs in each deployment site canister are then combined to enhance detection and each resulting sample is concentrated to ten microliters for HR GC/MS analysis.

In order to assess the potential of SPMDs to determine if mills are discharging dioxin, DEP has funded studies at the University of Maine Environmental Chemistry Laboratory (formerly the Water Research Institute) since 1999 through the Surface Water Ambient Toxics (SWAT) program. In 1999, the focus was development and refinement of field and laboratory techniques by deploying the SPMDs in the nearby Penobscot River for 3 one-month trials and then retrieving them for laboratory analysis.

In 2000, four studies or deployments were conducted as described below (Tables 4 and 5)and in more detail by Shoven (2001).

TABLE 4. Objectives of the 2000 Field Season Deployments

Objective	**#**	**# of SPMDs**
➢ Deployment Time Study: To determine SPMD uptake rates and biofouling over the 28-day deployment period. **Location: Androscoggin R. at Dixfield (10A,B)**	1, 2	20 SPMDs per deployment with 5 retrieved each week for 4 weeks

➢ Androscoggin Above/Below Study: To test the ability of SPMDs to detect differences in dioxin in the river Above/Below a mill. **Locations: Rumford Point (13) and Dixfield (10)**	4	20 SPMDs per site with all retrieved after 28 days
➢ Kennebec Above/Below Study: To test the ability of SPMDs to detect differences in the river Above/Below a mill. **Locations: Norridgewock (11) and Fairfield (12)**	3	10 SPMDs per site with all retrieved after the 54 days

TABLE 5. Descriptions of the 2000 Field Season Deployments

Deployment #	**Deployed**	**Retrived**	**Time (days)**	**Site**	**SPMDs per site**	**#SPMDs / sample**	**# Reps**
1	6/2/00	6/9/00	7	10-A	5	5	1
		6/16/00	14	10-B	5	5	1
		6/23/00	21	10-A	5	5	1
		6/30/00	28	10-B	5	1	5
2	7/7/00	7/14/00	7	10-A	5	5	1
	6/30/00	7/14/00	14	10-B	5	5	1
	7/7/00	7/28/00	21	10-A	5	5	1
	6/30/00	7/28/00	28	10-B	5	1	5
3	8/3/00	9/26/00	54	11	10	2	5
				12	10	2	5
4	9/19/00	10/17/00	28	10	20	2	10
				13	20	2	10

Results were as follows.

Deployment Time Study, Deployments 1 and 2

One objective was to determine differences in uptake in colder water (June) than in warmer water (July). Another objective was to determine if uptake rates were constant over time or if biofouling with growths of algae and accumulation of other materials would change the uptake rates. This is

critical to know to help determine the optimum length of deployment time. Longer deployment times should result in more uptake of dioxin unless biofouling or other processes reduce or eventually stop further uptake. For these and all deployments, SPMDs were suspended from floats so as to be approximately 1 meter below the water surface in all water levels at a location that was at least 4 m deep.

Results showed that uptake of TCDF continued over the 4 weeks in each month (Figure 1), as did uptake of many other furans as well (Table 6). No TCDD or PeCDD and only a few other dioxins were detected. The two curves show that uptake rates were considerably higher in warmer water (July) than in colder water (June)(Figure 1). The different slopes documented different uptake rates for each week for each deployment. In June uptake rates were relatively low for the first three weeks also likely reflecting lower temperatures during that period. Differences for all weeks may also be due to other factors including river velocities, dilution of dioxin levels in the river due to changes in river flow volume, suspended sediment load, dissolved organic carbon, and measurement error, among others.

Qualitatively, the biofouling on the membrane increased in coverage and changed characteristics over the four-week period progressing from tiny tan specs to larger army green, rod-like shapes. Each week the deployment canisters had more growth collected on the surfaces. Since uptake rates during week 4 was not diminished from earlier weeks in either month, biofouling did not seem to be an important factor in these 30 day exposures during June and July.

Kennebec Above/Below Study, Deployment 3

This study was conducted in conjunction with fish collections and caged mussel studies at the same two stations in order to be able to compare performance of all the studies in terms of MSDs for the above/below stations. This was a longer deployment than any of the others (Table 5). Results of deployment 3 show that TCDF was the most abundant congener detected (Figure 2). No TCDD nor any PeCDD or PeCDF were detected, but small amounts of other dioxins and furans were detected. Although TCDF appeared increased at Fairfield, the station below the SAPPI Somerset mill, the difference was not significant (error bars are 95% confidence limits). There were no significant differences in above/below concentrations for any other congener with the exception of OCDD, which was higher at the station above the mill. However, relatively small sample size (n=5) and considerable variation at each site (TCDF CV=24-40%, DTEo CV=26-29%) resulted in MSDs (105% for TCDF and 78% for DTEo) well above the target of 10%.

Figure 1.

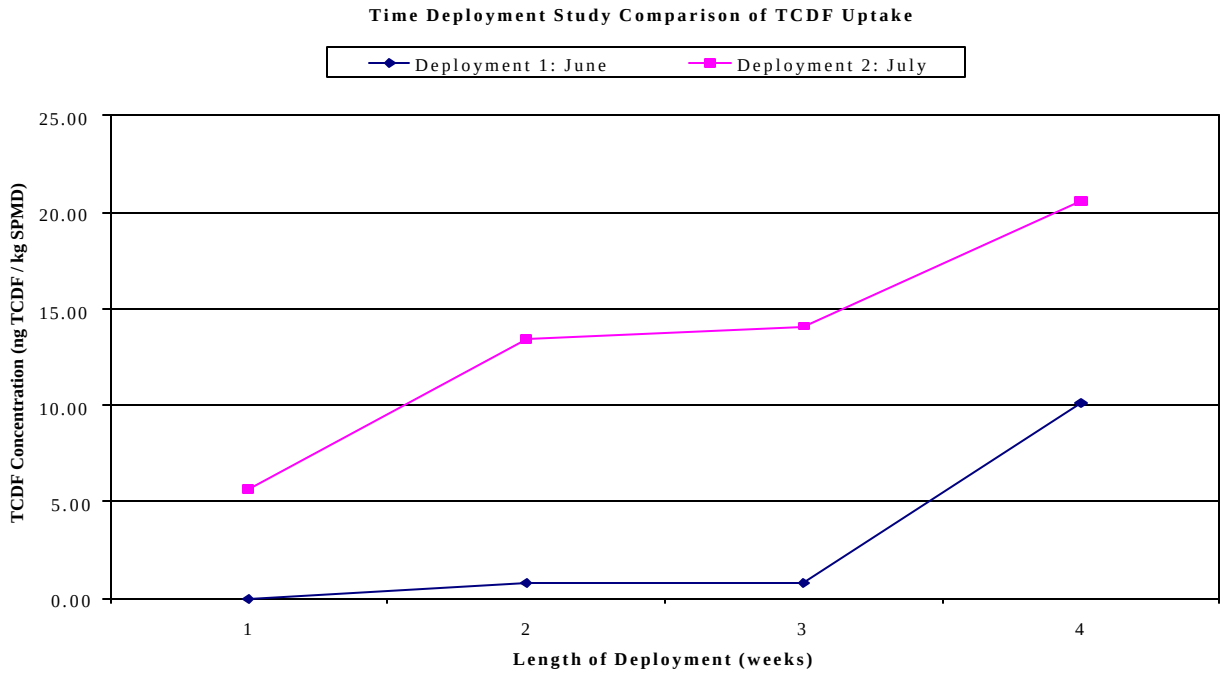
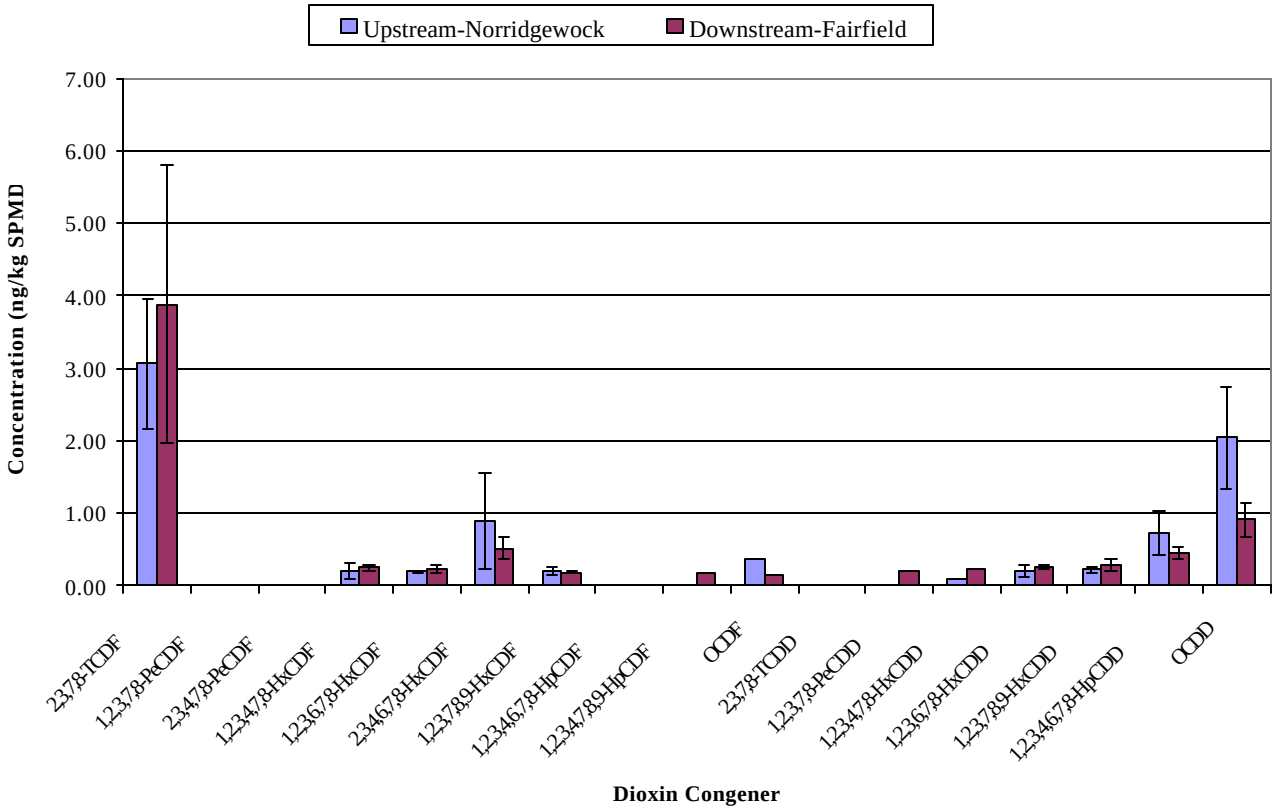


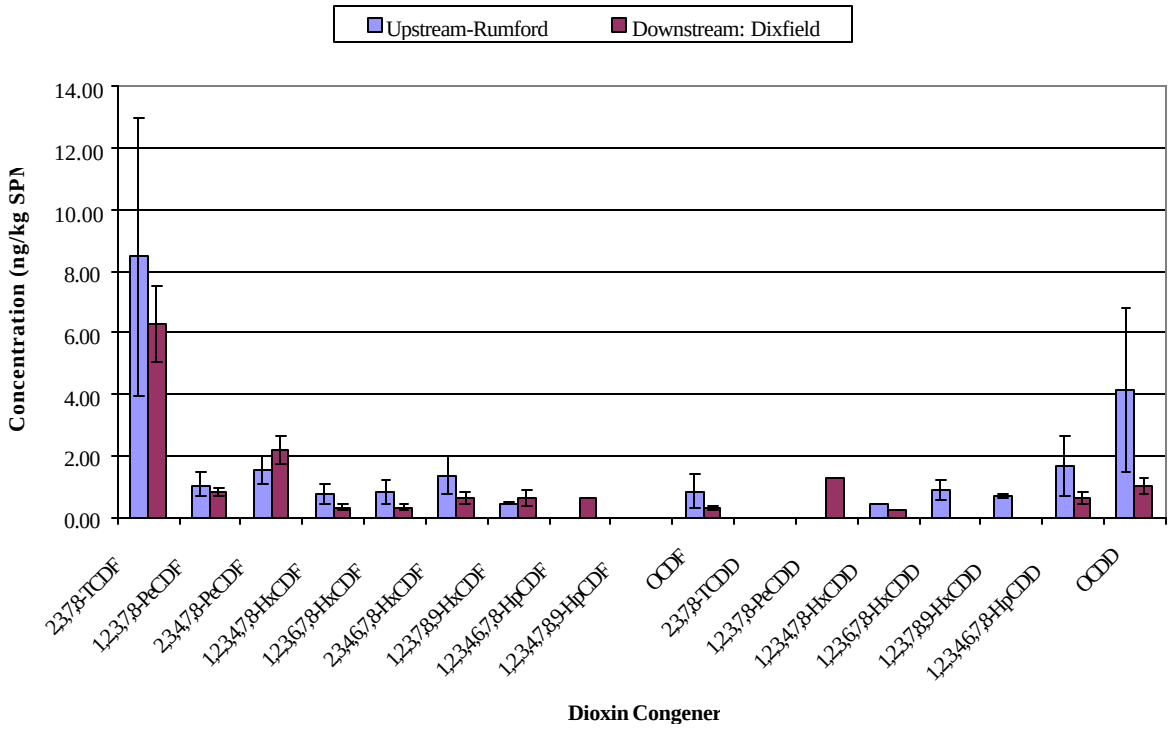
Figure 2. Kennebec River Upstream-Downstream Deployment



Androscoggin Above/Below Study, Deployment 4

Like the Kennebec study, TCDF was most abundant, but appeared slightly higher upstream of the mill, although the difference was not significant. No TCDD was detected but most other congeners were at one or both stations. There were no significant differences between the two stations for any congener with the exception of OCDD which was significantly higher upstream. Although sample sizes were higher (n=10) than for the Kennebec study (n=5), so was the variance (TCDF CV=28-75%, DTEo CV=45-79%) resulting in MSDs (77% and 129% for TCDF and DTEo respectively) that were similar to those from the Kennebec, also well above the target of 10%.

Figure 3. Androscoggin River Upstream-Downstream Deployment 4



Conclusions

Comparison of deployments 1,2 and 4 showed uptake of TCDF (mean=8.66+-6.33 ng/kg) in mid September-mid October deployment were lower, similar to those of June (mean=10.08+-0.62 ng/kg), than those of July (mean=20.6+-7.09 ng/kg) likely resulting from temperature differences. Therefore, for maximum uptake, July and August would be better months for use of SPMDs. Uptake rates were not
constant probably due to a number of factors, but bio- fouling did not seem to be the problem in 30 day exposures. Deployment 3, a 54 day exposure on the Kennebec River resulted in lower uptake than the other deployments, which is most likely due to lower levels of dioxins and furans in the Kennebec compared to the Androscoggin.

4.2

BROMINATED ORGANICS

BROMINATED ORGANICS

The use of polybrominated biphenyls (PBBs) and diphenyl ethers (PBDEs) as fire retardants has significantly increased in the past 25 years. These compounds, structurally similar to their chlorinated counterparts, are showing up in the environment in increasing numbers. As the levels of PCBs and DDT are decreasing, the levels of the brominated compounds are on the increase. These compounds are currently used in the plastic components of computers, televisions, circuit boards, fabric for seat cushions in cars and buses and in various other textiles.

PBDEs were first discovered in the environment in 1981 in pike from Sweden. Data presented at the Dioxin 99 meeting in Venice, Italy, showed increasing amounts of these fire retardants in steelhead trout from Lake Michigan. In the May 1, 2000 issue of Environmental Science and Technology, scientists in the European community are proposing curbing the use of these compounds. The production of PBBs have been voluntary phased out by manufacturers because of environmental issues. PBDEs are also being phased out and replaced by tetrabromobisphenol A (TBBPA).

This study consists of screening for these compounds using the extracted archives from 35 previous DMP/SWAT dioxin/coplanar PCB samples. The extraction methods are similar so it is likely that these compounds exist in the extracts if they were present in the original samples. We will composite 2-5 samples from each species and sampling area, add labeled surrogates and analyze them using high resolution MS against calibrated standards. The values would not be quantitative since the surrogates would not have gone through the entire extraction process however the presence of these compounds would be an indication of potential contamination. At sites that have PBBs identified, we could then analyze the archived extracts from the past three years to determine if there is a historical pattern per site.

This study has not yet been performed but will be modified and performed during 2002.

4.3

PCB IN HATCHERY FISH

PCB STUDY OF HATCHERY FISH

In Maine, game fish species analyzed for PCBs as part of the REMAP program in 1993-4 contained 5 to 190 ppb total PCBs in whole fish. Wild fish had a mean total PCB concentration of 12 ppb (N=17) whereas hatchery fish had mean total PCB concentration of 21 ppb (N=11). The Maine Bureau of Health's Fish Tissue Action Level is 11 ppb for filets. From previous studies a factor of ~6.5 was determined for the ratio of concentrations of PCB in whole brown trout to that in filets. Using this factor then the estimated concentrations of PCB in hatchery fish and wild fish were ~ 3 ppb and 2 ppb respectively. In 2000 the Biological Resources Division of the US Geological Survey informed DEP that Pennsylvania, hatchery-reared trout stocked into state waters were found to have total PCB concentrations of 30 to 400 ppb (ng/g). It seems that hatchery fish in Maine may not be as contaminated as those in Pennsylvania, but there is some evidence that hatchery fish may be higher in PCBs than wild fish of the same species. Consequently, we proposed to determine the PCB burden of the major fish species produced in Maine hatcheries for stocking in state waters, as well as the PCB content of the major sources of commercial diets fed to the fish.

The three most numerous fish reared for stocking in Maine are brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*) and landlocked Atlantic salmon (*Salmo salar*). We collected five spring yearlings of each species from one or more hatcheries just prior to scheduled stocking. The fish from each hatchery were skinned, filleted, and combined into a single composite sample for congener-specific analysis of total PCBs. A sample of the feed for each lot of fish was also collected for the same analysis.

The results of this preliminary study show that most samples exceeded the Maine Bureau of Health's Fish Tissue Action Level for PCB (11 ug/kg) (Table 4.3.1). The mean (26.7 ppb) was slightly higher than that found in the REMAP program. Concentrations appear to be higher in brown trout from the Casco hatchery and in landlocked salmon than in brown trout from other hatcheries or in brook trout. Concentrations in the feed were much higher but quite variable. Reasons for lower concentrations in the REMAP fish might include 1) depuration and 2) growth dilution. The fish from REMAP were captured from lakes and ponds where their natural food presumably has lower concentrations of PCBs than hatchery feed. Therefore, the fish may depurate with time. In addition, growth dilution would account for reduced concentrations if there is less intake in the wild. There was not a good correlation between fish concentrations and feed concentrations. Because these results are from a preliminary study and the feed datum are significantly different from values reported in other studies and analyses, the tissue and feed samples have been sent out for retesting to confirm the results. As well, this study will be repeated in 2002 to verify the nature and extent of these concentrations in tissue, feed, and additional work on sediment from hatchery settling ponds. This second study will begin to investigate initial findings on growth and depuration effects on PCB in tissue samples.

Table 4.3.1

Total PCB in fish and feed from DIFW hatcheries, 2000 (ppb)

hatchery		date	brook trout	brown trout	salmon	species	feed type
Casco							
	fish	04/20/2001		40.6	55.3	BNT	Corey 4 pt Vigor
	feed				1024	LLS	#3 Corey
Dry Mills							
	fish	04/01/2001	15.2				
	feed		1579			BKT	Shur Gain trout brood stock B2N 5G7
Emden							
	fish	04/20/2001			45.2		
	feed				2362	LLS	Corey Aqua transfer 3 lot 353080
type of feed							
Enfield							
	fish	04/19/2001	23.9				
	feed		240			BKT	Brook trout starter ration 3.5 mm B2N 6x8
brood fish			8.75				
brood fish eggs			25.1				
Grand Lake Stream							
	fish	05/03/2001			39.1		
	feed				694	LLS	Shur Gain 3.5 pt
New Gloucester							
	fish	04/20/2001		22.4			
	feed					BNT	Vigor 5 5212 ZBR
Palermo							
	fish	04/20/2001	4.94	11.2		BKT	Vigor #4 lot 520 ZBR
	feed		355			BNT	Vigor #4 lot 520 ZBR

4.4

CAGED MUSSEL DIOXIN BIOASSAY

CAGED MUSSEL DIOXIN BIOASSAY

This project was a cooperative one with the Maine Department of Inland Fisheries and Wildlife (DIFW) a Friends of Merrymeeting Bay (FOMB) assisted by a consultant, Applied Biomonitoring of Kirkland, Was Caged bivalves have been used to monitor pulp and paper mill effluents in Finland for over 20 years. Environment Canada is currently considering caged bivalves as an alternative to the required adult fish su their Environmental Effects Monitoring after several successful pilot studies. Caged bivalves are a poten powerful tool because of their ability to quantify exposure and effects over space and time. Caged bivalv an advantage of increased sample size over fish that are often difficult to collect in desired numbers. The size range can be also be standardized. This should limit dioxin variability in mussel tissues thereby allov smaller MSDs to be detected. Caged mussels anchored in place represent exposure at a fixed point in spa unlike fish which may move around.

The approach was to measure survival, growth, and bioaccumulation of dioxins and furans in caged fresh mussels at the same time and locations above and below the SAPPI Somerset bleached kraft pulp and pap on the Kennebec River, Norridgewock and FAIRFIELD, as the fish collections and SPMD studies, in orc compare uptake of contaminants and MSDs among all these Above/Below tests. Freshwater mussels, *Ell complanata*, were collected by SCUBA divers from DIFW and FOMB from Nequasset Lake, an undevel lake in Woolwich serving as Bath's water supply. The mussels were weighed, sorted by length, and then randomly distributed by length to nylon mesh bags that were then attached to PVC frames and enclosed v polypropylene mesh predator guards according to the methods of Salazar and Salazar (2000). An initial s of 5 composites of 35 mussels was collected and subsequently analyzed for all 2378- substituted dioxins furans, percent lipid and percent solids. Individual identities were noted by position within each mesh ba cages enabling calculation of survival and growth for each individual.

Ten cages of 35 mussels each were placed at both Norridgewock and Fairfield on August 3, 2000 and ret September 26, 2000, giving a 54 day exposure. Upon retrieval mussels were measured for length and we then shucked. Shell and soft tissues were then weighed. Tissues of mussels from each cage were composi one sample for analysis for all 2378- substituted dioxins and furans, percent lipid and percent solids. Ind mussels were also monitored for survival and growth.

Results of the initial 5 composite samples from Nequasset Lake showed no detectable dioxins or furans (4.4.1). This was interesting because feral fish from a number of other relatively undeveloped and somew developed lakes and ponds as well as rivers have always been found to contain measurable levels of TCD some other dioxins and furans. Nor at the end of the exposure did the mussels contain any measurable T either. Measurable concentrations of TCDF, however, were found in all samples at both stations, and ma dioxins and furans were found as well in most samples. Concentrations were similar to those in bass at Norridgewock but 2-3 x lower than those in bass at Fairfield on a wet weight basis, and similar to those in bass but higher than in small bass on a lipid weight basis at both stations. Concentrations were higher tha in suckers, sucker livers, and SPMDs on a lipid weight basis at both stations. MSDs were similar for TCI lower for DTEo to those of fish, but lower for TCDF and higher for DTEo than SPMDs (Table 4.4.2). Th no significant difference in TCDD, TCDF, or DTEo between the two stations, unlike the results for fish.

Table 4.4.1 Dioxin and furan in caged mussels (ppt)

		KNW1	KNW2	KNW3	KNW4	KNW5	
Compound							
2378-tcdf	0.11	0.52	0.31	0.62	0.47	0.33	
12378-pecdf	0.25	0.36	0.54	<DL	0.21	<DL	
23478-pecdf	0.25	<DL	<DL	<DL	<DL	<DL	
123478-hxcdf	0.25	0.33	0.41	<DL	0.20	<DL	
123678-hxcdf	0.25	<DL	<DL	<DL	0.17	<DL	
234678-hxcdf	0.25	<DL	<DL	<DL	<DL	<DL	
123789-hxcdf	0.25	1.02	0.75	0.41	0.26	0.51	
1234678-hpcdf	0.50	0.33	0.42	0.61	<DL	<DL	
1234789-hpcdf	0.50	<DL	<DL	<DL	<DL	<DL	
ocdf	0.50	<DL	1.05	<DL	<DL	<DL	
2378-tcdd	0.10	<DL	<DL	<DL	<DL	<DL	
12378-pecdd	0.25	<DL	0.35	<DL	<DL	<DL	
123478-hxcdd	0.25	<DL	<DL	<DL	<DL	<DL	
123678-hxcdd	0.25	0.51	<DL	0.36	<DL	0.26	
123789-hxcdd	0.25	0.15	0.22	0.34	<DL	<DL	
1234678-hpcdd	0.50	0.75	1.22	0.83	0.5	0.35	
ocdd	0.50	1.69	0.65	0.84	2.05	0.66	
DTEo		0.72	1.04	0.17	0.55	0.09	
DTEd		1.14	1.39	0.97	0.78	0.94	
% Lipids		0.52	0.63	0.57	0.49	0.59	

		KNW6	KNW7	KNW8	KNW9	KNW10	KNW ave
Compound							
2378-tcdf	0.11	0.19	0.36	1.15	0.28	1.06	0.45
12378-pecdf	0.25	<DL	0.31	0.61	0.25	0.42	
23478-pecdf	0.25	<DL	<DL	0.25	<DL	<DL	
123478-hxcdf	0.25	<DL	0.21	0.49	<DL	0.18	
123678-hxcdf	0.25	<DL	<DL	<DL	<DL	0.20	
234678-hxcdf	0.25	<DL	<DL	<DL	<DL	<DL	
123789-hxcdf	0.25	0.37	0.17	0.63	0.28	0.49	
1234678-hpcdf	0.50	<DL	0.25	0.36	0.51	0.19	
1234789-hpcdf	0.50	<DL	<DL	<DL	<DL	<DL	
ocdf	0.50	<DL	<DL	<DL	<DL	<DL	
2378-tcdd	0.10	<DL	<DL	0.10	<DL	<DL	<0.1
12378-pecdd	0.25	<DL	0.25	0.39	0.18	<DL	
123478-hxcdd	0.25	<DL	<DL	0.51	<DL	0.35	
123678-hxcdd	0.25	0.41	<DL	0.48	0.18	0.21	
123789-hxcdd	0.25	<DL	<DL	0.41	<DL	0.26	
1234678-hpcdd	0.50	0.69	1.06	1.35	0.51	1.14	
ocdd	0.50	0.48	0.69	1.51	0.72	0.61	
DTEo		0.06	0.52	1.14	0.38	0.89	0.51
DTEd		0.91	0.92	1.52	0.90	1.06	1.05
% Lipids		0.48	0.53	0.87	0.58	0.67	0.56

Table 4.4.2 Minimum Significant Differences for 2000 Above/Below test

STATIONS	SPECIES	N	TCDDw ppt	%bg	TCDFw ppt	%bg	DTEow ppt	%bg
FISH								
ARP/ARF	SMB	10	0.14	**280**	2.23	**384**	0.50	**526**
		20	0.10	**200**	1.58	**272**	0.35	**368**
KNW/KFF	SMB	10	0.17	**340**	0.53	**129**	0.2	**400**
		20	0.12	**240**	0.38	**93**	0.14	**280**
	sSMB	10	0.09	**180**	0.64	**139**	0.16	**267**
		20	0.06	**120**	0.45	**98**	0.11	**183**
	WHS	10	0.16	**320**	0.46	**164**	0.26	**520**
		20	0.11	**220**	0.32	**114**	0.18	**360**
PBW/PBL	SMB	10	0.09	**180**	0.15	**20**	0.15	**107**
		20	0.06	**120**	0.11	**15**	0.11	**79**
	WHS	10	0.31	**620**	0.88	**154**	0.39	**390**
		20	0.22	**440**	0.62	**109**	0.27	**270**
LIVERS								
KNW/KFF	WHS	10	1.23	**425**	13.31	**261**	2.87	**191**
		20						
MUSSELS								
KNW/KFF		10	<		0.57	**89**	0.69	**133**
		20	<		0.41	**64**	0.49	**94**
SPMDs								
KNW/KFF		5						
		10						
ARP/ARF		10						
		20						

STATIONS	SPECIES	N	TCDDL ppt	%bg	TCDFL ppt	%bg	DTEoL ppt	%bg
FISH								
ARP/ARF	SMB	10	19.6	**131**	189.7	**123**	57	**219**
		20	13.9	**93**	134.1	**87**	40.6	**156**
KNW/KFF	SMB	10	24.4	**176**	63.3	**71**	13.7	**127**
		20	17.2	**124**	44.8	**50**	9.7	**90**
	sSMB	10	1.49	**115**	8.7	**73**	2.3	**163**
		20	1.05	**81**	6.1	**51**	1.63	**116**
	WHS	10	2.57	**139**	8.4	**84**	5.5	**355**
		20	1.82	**98**	6	**60**	3.9	**252**
PBW/PBL	SMB	10	18.7	**117**	208.5	**95**	46	**41**
		20	13.2	**83**	147.5	**67**	32.5	**79**
	WHS	10	1.83	**153**	6.23	**48**	2.13	**107**
		20	1.3	**108**	4.76	**36**	1.5	**75**
LIVERS								
KNW/KFF	WHS	10	4.62	**453**	51.7	**272**	11.4	**193**
		20						
MUSSELS								
KNW/KFF		10	<		86.6	**78**	105	**119**
		20	<		61.2	**55**	74.5	**85**
SPMDs								
KNW/KFF		5	<		3.21	**105**	0.38	**78**
		10	<		2.27	**74**	0.26	**53**
ARP/ARF		10	<		6.5	**77**	1.89	**129**
		20	<		6.17	**73**	1.33	**90**

4.5

CAGED MUSSEL PCB STUDY

CAGED MUSSEL PCB STUDY

Previous SWAT studies have documented concentrations of PCBs in 3 species of freshwater fish from the Kennebec River below the former Edwards dam in Augusta that warrant a no consumption fish advisory. DEP has been trying to identify sources by analyzing sediments from the local area by use of an ELISA. Identification of sources by this method is limited by hydrological patterns of scouring and deposition. Areas where appropriate sediment can be found may not be adjacent to sources.

Caged bivalves have been used to monitor persistent bioaccumulative toxics such as PCBs for over 20 years. In southern California, caged bivalves were used to establish chemical gradients from suspected chemical sources for both DDT and PCBs. One advantage of caged bivalves is that they may be deployed at any location. We used caged mussels to supplement the current sediment approach for identify sources.

The proposed approach was to measure survival, bioaccumulation, and growth in caged freshwater mussels after *in situ* exposure to existing environmental conditions at 9 stations along a suspected longitudinal gradient of PCB contamination from above the former Edwards Dam to Merrymeeting Bay. Stations were selected to bracket potential existing or former industrial or municipal sources. A total of 3 cages were deployed in a transect across the river at each station. This facilitated identification of the hotspot area as well as define possible upstream and downstream boundaries or gradients. Cages of 20 mussels each were placed at each location along the transect for a period of 53 days. Tissues were pooled from each cage to establish one sample per cage to determine mean concentrations of PCBs, percent lipid and percent solids at the different sites (or to establish the gradient). Individual mussels were monitored for survival and growth. An initial sample of 5 composites of 35 mussels was analyzed for PCBs, percent lipid and percent solids. The study was coordinated with the Maine Department of Inland Fisheries and Wildlife, Friends of Merrymeeting Bay, and Applied Biomonitoring.

Results show that the highest concentrations were at the middle of the river at station 5, below the Augusta POTW outfall, and at station 6 west, below Hallowell (Table 4.5.1). These potential sources need to be investigated further to determine if there are continuing sources. These high values may also represent historical discharges. Sediment contamination from these two areas should be investigated. There was some variation along transects at most of the stations with the highest values not always where expected. Highest values at station 2 below Riggs Brook, which flows by the O'Connor PCB contaminated site on Route 17, were on the opposite (W) side of the river from the confluence with Riggs Brook. The highest value at station 3 below the former Edwards mill was in the middle of the river, where most of the flow goes. At the next station, 4, below Ft Western, which has been rumored to have had electrical transformers stored in a former warehouse on site, concentrations were low at all locations along the transect. At station 7 below Gardiner, the highest value was across the river on the east side. These results may represent the effects shifting location of the main current distributing the discharges from the potential sources across the river or indicate other unknown sources.

Survival was 95-100% at all stations. Mussels at all stations, except station 5 below Augusta, had significant increases in tissue weight during the exposure. Low tissue weights and low growth at station 5 middle and station 6 west were coincident with the highest PCB concentrations.

More details of this study and of the Caged Mussel Dioxin Bioassay may be seen in the final report from the consultant, Applied Biomonitoring, available with this 2000 SWAT report separately at http://www.state.me.us/dep/blwq/monitoring.htm The conclusions of that report are solely those of Applied Biomonitoring, and not necessarily those of DEP.

Table 4.5.1 PCB concentrations in caged mussels in the Kennebec River (ppb)

Station	East	Middle	West	Location
1	18.4	25.8	29.5	above Riggs Brook
2	18.4	26.9	45.8	below Riggs Brook
3	2.7	54.8	3.9	below Edwards mill
4	4.3	6.1	3.0	below Ft Western
5	16.5	188	31.5	below Augusta POTW
6	61.2	35.9	125	below Hallowell
7	50.3	6.6	24.8	below Gardiner
8	20.1	26.9	lost	below Gardiner POTW
9	64.2	16.7	lost	below Richmond

DEP ID#		DL	PC-01-08	PC-01-11	PC-01-15	PC-02-02	PC-02-26	PC-02-29
EXT ID#		ug/kg dw	1441	1445	1444	1426	1446	1427
Analytes	IUPAC#							
2,4'-Dichlorobiphenyl	8	1.0	0.639	0.360	0.480	0.561	0.400	0.761
2,2',5-Trichlorobiphenyl	18	1.0	<DL	<DL	0.360	<DL	<DL	<DL
2,4,4'-Trichlorobiphenyl	28	1.0	<DL	<DL	<DL	<DL	<DL	<DL
2,4,5-Trichlorobiphenyl	29	1.0	<DL	0.880	<DL	0.641	0.760	0.361
2,2',3,5'-Tetrachlorobiphenyl	44	1.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',4,6-Tetrachlorobiphenyl	50	1.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',5,5'-Tetrachlorobiphenyl	52	1.0	<DL	<DL	<DL	<DL	<DL	<DL
2,3',4,4'-Tetrachlorobiphenyl	66	1.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',3,4,5'-Pentachlorobiphenyl	87	2.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',4,5,5'-Pentachlorobiphenyl	101	2.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',4,6,6'-Pentachlorobiphenyl	104	2.0	<DL	<DL	0.480	<DL	0.320	<DL
2,2',3,3',4,4'-Hexachlorobiphenyl	128	2.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',3,4,4',5'-Hexachlorobiphenyl	138	2.0	0.599	<DL	0.480	<DL	1.001	0.801
2,2',4,4',5,5'-Hexachlorobiphenyl	153	2.0	1.159	0.640	<DL	<DL	<DL	0.921
2,2',4,4',5,6'-Hexachlorobiphenyl	154	2.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',3,4',5,5',6-Heptachlorobipheny	187	2.0	0.559	<DL	<DL	<DL	<DL	0.120
2,2',3,4',5,6,6'-Heptachlorobipheny	188	2.0	3.357	1.680	0.480	7.813	5.643	<DL
2,2',3,3',4,4',5,6-Octachlorobipheny	195	3.0	0.200	0.160	<DL	<DL	<DL	0.521
2,2',3,3',4,5',6,6'-Octachlorobipher	200	3.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',3,3',4,4',5,5',6,6'-Decachlorobi	209	5.0	<DL	<DL	<DL	<DL	<DL	<DL
Total PCBs			29.5	25.8	18.4	45.8	26.9	18.4
Sample weight (g, dry weight)			25.0257	25.0029	25.0022	24.959	24.9861	24.9616
Surrogate Recovery		% rec (65-1	91.0	88.2	98.3	103	70.4	80.0

The tissue blank is an oil matrix.
Values below the detection limit are estimated values and should be considered qualitative.
They are provided for information only.

DEP ID#		DL	PC-03-06	PC-03-21	PC-03-30	PC-04-12	PC-04-13	PC-04-19
EXT ID#		ug/kg dw	1074	1075	1076	1077	1078	1079
Analytes	IUPAC#							
2,4'-Dichlorobiphenyl	8	1.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',5-Trichlorobiphenyl	18	1.0	<DL	0.505	0.291	0.453	0.313	0.169
2,4,4'-Trichlorobiphenyl	28	1.0	<DL	0.269	<DL	<DL	<DL	<DL
2,4,5-Trichlorobiphenyl	29	1.0	<DL	24.020	<DL	<DL	<DL	<DL
2,2',3,5'-Tetrachlorobiphenyl	44	1.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',4,6-Tetrachlorobiphenyl	50	1.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',5,5'-Tetrachlorobiphenyl	52	1.0	<DL	<DL	<DL	<DL	<DL	<DL
2,3',4,4'-Tetrachlorobiphenyl	66	1.0	<DL	<DL	<DL	0.113	0.267	<DL
2,2',3,4,5'-Pentachlorobiphenyl	87	2.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',4,5,5'-Pentachlorobiphenyl	101	2.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',4,6,6'-Pentachlorobiphenyl	104	2.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',3,3',4,4'-Hexachlorobiphenyl	128	2.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',3,4,4',5'-Hexachlorobiphenyl	138	2.0	0.123	<DL	<DL	<DL	<DL	<DL
2,2',4,4',5,5'-Hexachlorobiphenyl	153	2.0	0.163	<DL	<DL	<DL	<DL	<DL
2,2',4,4',5,6'-Hexachlorobiphenyl	154	2.0	<DL	<DL	<DL	<DL	<DL	0.626
2,2',3,4',5,5',6-Heptachlorobipheny	187	2.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',3,4',5,6,6'-Heptachlorobipheny	188	2.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',3,3',4,4',5,6-Octachlorobipheny	195	3.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',3,3',4,5',6,6'-Octachlorobipher	200	3.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',3,3',4,4',5,5',6,6'-Decachlorobi	209	5.0	<DL	<DL	<DL	<DL	<DL	<DL
Total PCBs			3.9	54.8	2.7	3.0	4.3	6.1
Sample weight (g, dry weight)			24.4869	29.7253	27.5164	26.4663	21.1224	27.061
Surrogate Recovery	% rec (65-1		111	121	65.2	73.0	81.6	79.8

The tissue blank is an oil matrix.
Values below the detection limit are estimated values and should be considered qualitative.
They are provided for information only.

DEP ID#		DL	PC-05-09	PC-05-18	PC-05-27	PC-06-03	PC-06-14	PC-06-23
EXT ID#		ug/kg dw	1436	1437	1442	1443	1431	1429
Analytes	IUPAC#							
2,4'-Dichlorobiphenyl	8	1.0	<DL	1.398	0.400	0.759	3.078	1.202
2,2',5-Trichlorobiphenyl	18	1.0	<DL	5.353	0.280	<DL	<DL	<DL
2,4,4'-Trichlorobiphenyl	28	1.0	<DL	<DL	<DL	<DL	<DL	<DL
2,4,5-Trichlorobiphenyl	29	1.0	1.439	2.037	<DL	1.438	1.159	<DL
2,2',3,5'-Tetrachlorobiphenyl	44	1.0	<DL	2.557	<DL	<DL	0.120	<DL
2,2',4,6-Tetrachlorobiphenyl	50	1.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',5,5'-Tetrachlorobiphenyl	52	1.0	<DL	<DL	0.600	<DL	<DL	<DL
2,3',4,4'-Tetrachlorobiphenyl	66	1.0	<DL	0.959	<DL	<DL	<DL	<DL
2,2',3,4,5'-Pentachlorobiphenyl	87	2.0	<DL	<DL	0.200	0.200	0.160	<DL
2,2',4,5,5'-Pentachlorobiphenyl	101	2.0	<DL	0.360	<DL	<DL	<DL	0.120
2,2',4,6,6'-Pentachlorobiphenyl	104	2.0	<DL	0.160	<DL	<DL	<DL	<DL
2,2',3,3',4,4'-Hexachlorobiphenyl	128	2.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',3,4,4',5'-Hexachlorobiphenyl	138	2.0	<DL	0.439	1.280	1.239	1.279	6.891
2,2',4,4',5,5'-Hexachlorobiphenyl	153	2.0	0.719	0.320	4.401	<DL	<DL	2.243
2,2',4,4',5,6'-Hexachlorobiphenyl	154	2.0	1.199	<DL	<DL	<DL	<DL	0.401
2,2',3,4',5,5',6-Heptachlorobipheny	187	2.0	7.193	55.248	0.520	<DL	2.079	1.162
2,2',3,4',5,6,6'-Heptachlorobipheny	188	2.0	<DL	4.314	1.080	<DL	0.160	1.402
2,2',3,3',4,4',5,6-Octachlorobipheny	195	3.0	<DL	0.200	2.160	<DL	<DL	<DL
2,2',3,3',4,5',6,6'-Octachlorobipher	200	3.0	<DL	<DL	<DL	<DL	<DL	<DL
2,2',3,3',4,4',5,5',6,6'-Decachlorobi	209	5.0	<DL	<DL	<DL	<DL	<DL	<DL
Total PCBs			31.5	188.0	16.5	125.0	35.9	61.2
Sample weight (g, dry weight)			25.026	25.0328	24.9955	25.0272	25.0177	24.9617
Surrogate Recovery		% rec (65-1	92.5	122	72.1	76.6	95.5	70.4

The tissue blank is an oil matrix.
Values below the detection limit are estimated values and should be considered qualitative.
They are provided for information only.

DEP ID#		DL	PC-07-10	PC-07-17	PC-07-25	PC-08-05	PC-08-22	PC-09-01	PC-09-07
EXT ID#		ug/kg dw	1435	1432	1433	1430	1425	1428	1434
Analytes	IUPAC#								
2,4'-Dichlorobiphenyl	8	1.0	1.478	0.440	1.082	0.719	0.518	1.361	0.399
2,2',5-Trichlorobiphenyl	18	1.0	<DL	<DL	<DL	<DL	<DL	<DL	<DL
2,4,4'-Trichlorobiphenyl	28	1.0	<DL	<DL	<DL	<DL	<DL	<DL	<DL
2,4,5-Trichlorobiphenyl	29	1.0	<DL	0.400	<DL	0.958	2.073	2.922	0.599
2,2',3,5'-Tetrachlorobiphenyl	44	1.0	0.320	<DL	<DL	<DL	<DL	0.280	<DL
2,2',4,6-Tetrachlorobiphenyl	50	1.0	<DL	<DL	<DL	<DL	<DL	<DL	<DL
2,2',5,5'-Tetrachlorobiphenyl	52	1.0	<DL	<DL	<DL	<DL	<DL	<DL	<DL
2,3',4,4'-Tetrachlorobiphenyl	66	1.0	<DL	<DL	<DL	<DL	<DL	<DL	<DL
2,2',3,4,5'-Pentachlorobiphenyl	87	2.0	<DL	<DL	<DL	0.200	0.159	<DL	<DL
2,2',4,5,5'-Pentachlorobiphenyl	101	2.0	<DL	<DL	<DL	<DL	<DL	<DL	<DL
2,2',4,6,6'-Pentachlorobiphenyl	104	2.0	0.120	<DL	<DL	0.200	<DL	<DL	<DL
2,2',3,3',4,4'-Hexachlorobiphenyl	128	2.0	<DL	<DL	<DL	<DL	<DL	<DL	<DL
2,2',3,4,4',5'-Hexachlorobiphenyl	138	2.0	<DL	<DL	1.883	1.158	<DL	4.763	0.878
2,2',4,4',5,5'-Hexachlorobiphenyl	153	2.0	0.839	<DL	<DL	<DL	0.120	<DL	0.359
2,2',4,4',5,6'-Hexachlorobiphenyl	154	2.0	<DL	<DL	<DL	0.280	0.439	0.320	0.439
2,2',3,4',5,5',6-Heptachlorobiphe	187	2.0	6.633	<DL	1.803	0.359	0.120	4.282	0.758
2,2',3,4',5,6,6'-Heptachlorobiphe	188	2.0	1.638	0.240	0.481	0.240	1.675	0.360	0.399
2,2',3,3',4,4',5,6-Octachlorobiphe	195	3.0	0.240	0.120	0.200	0.240	<DL	0.160	<DL
2,2',3,3',4,5',6,6'-Octachlorobiph	200	3.0	<DL	<DL	<DL	<DL	<DL	<DL	<DL
2,2',3,3',4,4',5,5',6,6'-Decachloro	209	5.0	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Total PCBs			50.3	6.6	24.8	20.1	26.9	64.2	16.7
Sample weight (g, dry weight)			25.0274	24.9961	24.9641	25.0409	25.0821	24.9865	25.05
Surrogate Recovery	% rec (65-		108	83.8	79.0	74.3	103	66.2	66.0

The tissue blank is an oil matrix.
Values below the detection limit are estimated values and should be considered qualitative.

4.6

EEL STUDY

EEL STUDY

There are two principle fisheries for adult eels in Maine, a river fishery and a lake fishery. Most of the ee sold outside Maine in US and international markets, although some are consumed in Maine. People fishi need permits from either DMR or DIFW. DMR also funds several eel research projects at the University Maine. Limited data from previous years show that eels from rivers are often among the species most hig contaminated with a number of contaminants. Contaminant levels in eels from lakes are unknown. In 19 were captured from 3 lakes. In 1998 and 1999 we tried to get eels from 3 rivers as well, but were success partially in one river. Therefore, in 2000, we attempted to work with commercial eel fishermen to collec from each of three river, but were successful in collecting eels only from the Penobscot River below Bang fish were analyzed as four composites of five fish each for PCBs. Results show a high concentrations (m 253 ppb) of PCB well above the Maine Bureau of Health Fish Tissue Action Level (Table 3.1.1.1 Rivers This concentration is much higher than that from other species from the Penobscot River from previous S studies in 1994 and 1996.

4.7

XENOESTROGENS (from 1999)

Progress Report
Project # 2000625

Department of Environmental Protection

Investigation of the Estrogenic Potential of Agrochemicals and their Effect on the Atlantic Salmon (*Salmo salar*)

20 March 2002

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Introduction

Numerous toxicants of natural and anthropogenic origin have been released into the environment in quantities sufficient to disrupt developing endocrine and nervous systems in wildlife and humans (Colborn and Clement, 1992). Many such toxicants have been identified as acute problems in Maine, including organophosphates and other pesticides, herbicides, organo-arsenic, organo-mercury, dioxins and polychlorinated biphenyls (PCBs). These chemicals are especially harmful during embryonic, fetal and early post-natal periods because they may mimic or interfere with hormones, neurotransmitters, growth factors and other signaling molecules that normally control developmental processes. For mammals, gestational exposure to toxicants reflects the lifetime of maternal exposure before pregnancy. Exposure occurs all during prenatal and early post-natal development because the chemicals are accumulated in maternal fat stores. In egg-laying species, the most critical exposure period is just prior to ovulation. Exposure during development may result in organizational and irreversible changes. Consequences of endocrine disruption can be profound because of the pivotal role that hormones play in controlling development and reproduction (Colborn and Clement, 1992; Birnbaum, 1994). The endocrine system is enormously complex; a single chemical can induce alterations through multiple mechanisms.

The Narraguagus River is one of seven Maine rivers populated by native Atlantic salmon (*Salmo salar*). Surveys conducted by the Atlantic Salmon Commission have found that 40-50% of the stocked Atlantic salmon never leave the river to go to sea. This suggests that these fish may not have successfully completed the smoltification process, the physiological transition from a freshwater to a saltwater dwelling fish. Of the fish that do smolt and leave for the ocean, less than 1% of the originally stocked fish ever return to spawn (Beland, *personal comm.*). This represents an extremely high mortality of both pre-smolt and mature Atlantic salmon, and the current numbers of returning salmon cannot sustain a viable population. The reason for this mortality is unknown. One hypothesis is exposure to agrochemicals introduced into the watershed from runoff.

Nineteen agricultural chemicals are currently registered for use in maintaining blueberry fields of Maine (**Table IA and B**). The Narraguagus River in Eastern Maine runs through many of these blueberry fields, and is therefore potentially exposed to these chemicals through the watershed. Certain environmental contaminants can mimic the action of hormones and function as endocrine disrupters. These have been shown to disrupt normal processes of growth, differentiation and reproduction in many organisms. Very little is known about the effects of these agrochemicals on Atlantic salmon populations. Madsen *et al.* (1977) reported delay the onset of smolting in Atlantic salmon exposed to 17 ß-estradiol or 4-nonylphenol. The target appears to be the gill Na^+/K^+ATPase. At present, the mechanism of action is unknown, although evidence has indicated that the effect may be indirect via the central neuroendocrine system. Both cortisol and growth hormone production in salmonids are inhibited by estradiol (Young, 1996).

It is important to determine the estrogenicity of the pesticides, herbicides and fungicides that are used in the area of the Narraguagus River, since this may provide information on the cause of the Atlantic salmon population decline. These data may provide insight into possible

mechanisms of action used by xenoestrogens and their biological effects on this important sport fish.

Four of the chemicals used on Maine blueberry fields (hexazinone, diazinon, malathion and methoxychlor) have previously been tested for estrogenicity using the E-SCREEN test (Soto *et al.*, 1995). Only methoxychlor tested positive. These four chemicals are the active ingredients of several pesticides and herbicides. There are no data available on the estrogenicity of the formulation actually applied to the fields. In addition, no data exist on the biological effects of the other eight active components of herbicides/pesticides used in Maine (guthion, benomyl, phosmet, glyphosate, propiconazole, sethoxidim, clethodim and fluazifop-p-butyl). The degree of estragenicity of these twelve chemicals relative to 17 â-estradiol will be determined using E-SCREEN (Soto *et al*., 1995). The E-SCREEN test is based on two premises: (1) that a protein inherent in serum specifically inhibits proliferation of human estrogen-sensitive cells (MCF-7 cells, a human breast-cancer derived cell line; Soto *et al.*, 1995); (2) that estrogens (or compounds that mimic estrogen) induce cell proliferation by overriding the inhibitory effect.

Objectives

The long-term goal of our investigations is to determine whether exposure to agrochemicals affects the ability of the Atlantic salmon to successfully complete smoltification, enabling them to make the transition from freshwater to sea water. The specific aims addressed in this proposal were:

(1). To identify what chemicals are present in the water and sediments from selected Maine rivers.

(2). To determine if these chemicals have estrogenic activity using the E-SCREEN assay which measures proliferation of estrogen-responsive MCF-7 cells.

Materials and Methods

(1) Identification of Agrochemicals It has been established that Velpar (active ingredient, hexazinone) is present in the Narraguagus River all year (Haines, 2000). It was expected that other agrochemicals would also be detected in the river using GC/MS or high resolution GC/MS. Sediments were collected in borosilicate bottles with teflon caps from the Narraguagus River at three locations (Cherryfield, Deblois and Beddington) and stored at 4°C until extracted. Approximately 5 g of sediment was mixed, shaking, with an acetonitrile/water mixture (70:30, v/v) for 19 hrs. Agrochemical standards were prepared using serial dilutions in methanol. High (2.0ppb) and low (0.5 ppb) spikes were made. Standards were diluted to ~2.5 ppm in acetone/acetonitrile for use as standards on the GC/MS. Standards were run at the Sawyer Environmental Laboratory (University of Maine, Orono, ME). These included: azenphos-methyl, malathion, diazinon, methoxychlor, fluazifop-*p*-butyl, phosmet, hexazinone (active ingredient in Velpar), propiconazole and sethoxydim.

(2) E-Screen Assay A human breast cancer cell line (MCF-7) and the protocols for maintaining the cells and running the E-SCREEN were kindly provided by Drs. Ana Soto and Carlos Sonnenschein (Tufts University, Boston, MA). The cells were maintained in Dulbeccos Modified

Eagle Medium (GIBCO, Grand Island, NY) supplemented with 5% fetal bovine serum (GIBCO) in an atmosphere of 6.7% CO_2 under saturating humidity, at 37°C. Purified active ingredients were obtained from EPA repositories by Brian Perkins (University of Maine). All formulations applied in the field were provided by Dr. David Yarborough (Extension Blueberry Specialist, University of Maine). The 17 ß-estradiol was purchased from Sigma Chemical Co. (St. Louis, MO).

MCF-7 cells were plated at a concentration of 30,000-40,000 cells/well. The test compound was added directly to the medium, at different concentrations and incubated at 37° C for 5 days. Scoring of the estrogenic effects of each xenobiotic was done by first measuring the proliferative effect (PE), which is the ratio between the highest cell yield counted with the test chemical to the negative control (Soto *et al*., 1995). PE was then used to calculate the *relative proliferative effect* (RPE; *i.e.*, 100 times the ratio of the highest cell yield exposed to test chemical compared to estradiol, arbitrarily set at 100% (Soto *et al*., 1995). An RPE of 100% or greater indicates a full xenoestrogen, while a RPE score less than 100% indicates a partial xenoestrogen. A score close to zero indicates no estrogenic activity. These experiments will be repeated to enable us to perform statistical analysis. Details are given below.

Maintaining cell cultures - Cells were grown in 25cm^2 flasks with 5ml DMEM Dulbecco's Modified Eagle Medium) in 5% FBS with a media change every 3-4 days. Cells were at 90% confluency (~every 6-7 days) into 2 new flasks, using 100-200 ìl of cells and 5 ml media into each new flask. Cells were passed three times prior to the assay.

Dosing - Testing media was added 24 hours (+/- 3 hours) after subculturing cells. Growth media was removed, cells were rinsed and 1ml of CD FBS 5% experimental media was added to each well (DMEM without phenol red, with charcoal/dextran stripped FBS). Test chemicals were added, in three replicates, at 10nM, 1nM, 0.1nM, 10pM, 1pM. Cells were harvested on Day 5 after treatment.

Harvesting - Experimental media was aspirated, cells detached from plate by trypsinization and counted using a hemacytometer. A standard curve using estradiol was run in parallel with test samples

Results

Identification of Agrochemicals No pesticides were detected in sediment samples. Sediments were re-sampled and are awaiting analysis.

E-screen for estrogenic activity Compounds (analytical/pure) that have been tested and their RPEs are reported in **Table II**. Growth curves are shown in **Figs 1-4.** Those with estrogenic activity include methoxychlor, propiconizol, and dichlorophenoxyacetic acid (2,4-D). Since the active analytical compounds are applied in the field as mixtures with "inert" ingredients (such as surfactants), the analysis was repeated, using the formulations that were actually applied in the field. A comparison of the relative proliferative effects (RPEs) of the formulations to analytical compounds (at the percentage of active ingredient in formulation, % used in applying to field and full strength) is summarized in **Table III**. Orbit (active ingredient, 41.8% propiconizol) had an RPE of 86-93%. The RPE of Velpar (24-26%) was lower than its active ingredient, hexazinone (42-47%), suggesting that something in the formulation was inhibitory.

Discussion

2,4-D is a member of the chlorophenoxy compounds that act as broad-spectrum herbicides. During World War II, considerable effort was put toward their development, both to increase food production and as possible use in chemical warfare (Claassen, 2001). These compounds have been in continuous use since 1947. Their use has declined significantly in recent years primarily due to concerns over the presence of toxic contaminating compounds (*e.g*., 2,3,7,8 tetrachlorodibenzo-*p*-dioxin, TCDD). 2,4-D mimics the action of auxins, growth-stimulating plant hormones. No hormonal activity has been reported in animals, although the mechanism of toxicity is still poorly understood. There is an extensive, and often contradictory, database on the toxicity of chlorophenoxy chemicals to mammals (Claassen, 2001). The carcinogenicity of 2,4-D containing formulations has been controversial, confounded by the presence of TCDD in many commercial preparations. The carcinogenicity of analytical grade 2,4-D has not been yet been tested in rodents.

Studies in our laboratory have shown that laboratory exposures of softshell clams (*Mya arenaria*) to 2,4-D result in a dose-dependent effect on gonadal maturation. Exposed animals do not develop mature gametes as compared to controls. These studies are currently being repeated. Data reported here suggest that 2,4-D has relatively high estrogenic activity *in vitro* in the MCF-7 breast cancer cell line. Taken together, our studies suggest that 2,4-D has possible hormonal effects in animals, and warrant further investigation.

Methoxychlor has been shown previously to possess estrogenic activity in the E-SCREEN assay with a RPE reproduced in our laboratory (Soto *et a.l,* 1995). Methoxychlor, a DDT analog, is a member of the family of dichlorordiphenylethane pesticides. Symptoms of acute toxicity in humans and animals include fatigue and lethargy. Chronic exposures result in alterations in EEG patterns and varied reproductive effects. Studies of methoxychlor toxicity in the mouse have revealed problems in initiating and maintaining pregnancy, alterations in the development of preimplantation embryos and estrogenic effects on the oviduct and uterus. (reviewed in Claassen, 2001).

Propiconizol is a fungicide often used in control of fungal diseases of turfgrass.

Work remaining

Future work includes repeating assays of those compounds that were positive (methoxychlor, 2,4 D, and propiconizole). Assays will also be done on analytical compounds that have just been received (benomyl, glyphosate, and carbendazim). In addition, the following formulations and their active chemicals will be tested: Benlate (benomyl), Diazinon, Imidan (phosmet), Round Up (glyphosate), and Select 2 (Clethodim). We are also attempting to obtain Marlate (methoxychlor), Sinbar (terbacil), Cythion (malathion), Sethoxydim, and Fluazifop-*p*-butyl. In addition to being able to screen individual chemicals, the E-SCREEN assay can also be used to test mixtures of chemicals. Soto *et al.* (1994) have shown that estrogenic chemicals may act in a cumulative fashion. Compounds found to possess estrogenic activity *in vitro* will be further investigated *in vivo*, using fish models.

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Table IA: Analytical Compounds tested by E-SCREEN

Analytical Compound	manufacturer	Use*	E-SCREEN # of assays
clethodim	Valent	H	1
diazinon	Syngenta	I	1
fluazifop-p-butyl	Aeneca	H	-
hexazinone	DuPont	H	2
malathion	Cheminova	I	2
methoxychlor	Kincaid enterprises	I	4
phosmet	Zeneca	I	1
propiconizol	Syngenta	D	1
sethoxydim	BASF	H	1
terbacil	DuPont	H	-
(*not yet obtained*)			
benomyl		D	
glyphosate		H	
carbendazim		H	
2,4-D		H	

* H, herbicide; I, insecticide; D, disease control.

Table IB: Formulations used in Blueberry Culture

Formulation Compound	Active Ingredient	% Active Ingredient	Manufacturer	%/dilution in field	# [1]Assays
Benlate	benomyl	50%	DuPont		
Diazinon	diazinon				
Imidan 25EC	phosmet				
Imidan 70W	phosmet	70%	Gowan		
Round Up	glyphosate	41%	Monsanto		
Select 2	clethodim	25-27%	Valent		
Velpar	hexazinone	25%		5%	1
Orbit	propiconizol	41.8%	Syngenta	1:900	1
Super BK32	2,4-D[2]	16%	Agway		
	2 (2,4)-D p[3]	16%			
(*not yet obtained*)					
Marlate	methoxychlor				
Sinbar	terbacil				
Cythion	malathion				
Poast	sethoxydim, fluazifop-*p*-butyl				

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[1] limited information available; [2] 2,4- dichlorophenoxyacetic acid; [3]2 (2,4) dichlorophenoxy propionic acid

Table II: RPE values for Purified Test Compounds

Compound	Usage	RPE
Methoxychlor	Insecticide	57% 26% 38% 64%
Malathion	Insecticide	25% 22%
Hexazinone	Weed control	14%
Diazinon	Insecticide	12%
Clethodim	Weed control	20%
Phosmet	Insecticide	17%
Sethoxydim	Weed control	31%
Propiconizol	disease control	80% * 73% *
2,4-Dichlorophenoxy -acetic acid	weed control	91% * 66%*
2,4-Dichlorophenoxy -propionic acid	weed control	91% * 45% 14%

Table III Comparison of E-SCREEN results of formulations and analytical compounds

Formulation	Active ingredient	RPE
Velpar	(hexazinone, 25%)	26%
		24%
	hexazinone 25%[1]	47%
	hexazinone 5%[2]	42%
Orbit	(propiconizole, 41.8%)	93%
		86%
	propiconizole 41.8%[1]	92%
		65%
Super BK32	(16% 2,4D-acetic &	52%
	16% 2,4D propionic acid)	27%
	2,4D Acetic 16%[1]	42%
		30%
	2,4D Propionic 16%[1]	43%
		8%

[1] the percentage of active ingredient in formulation
[2] the percentage used in field applications

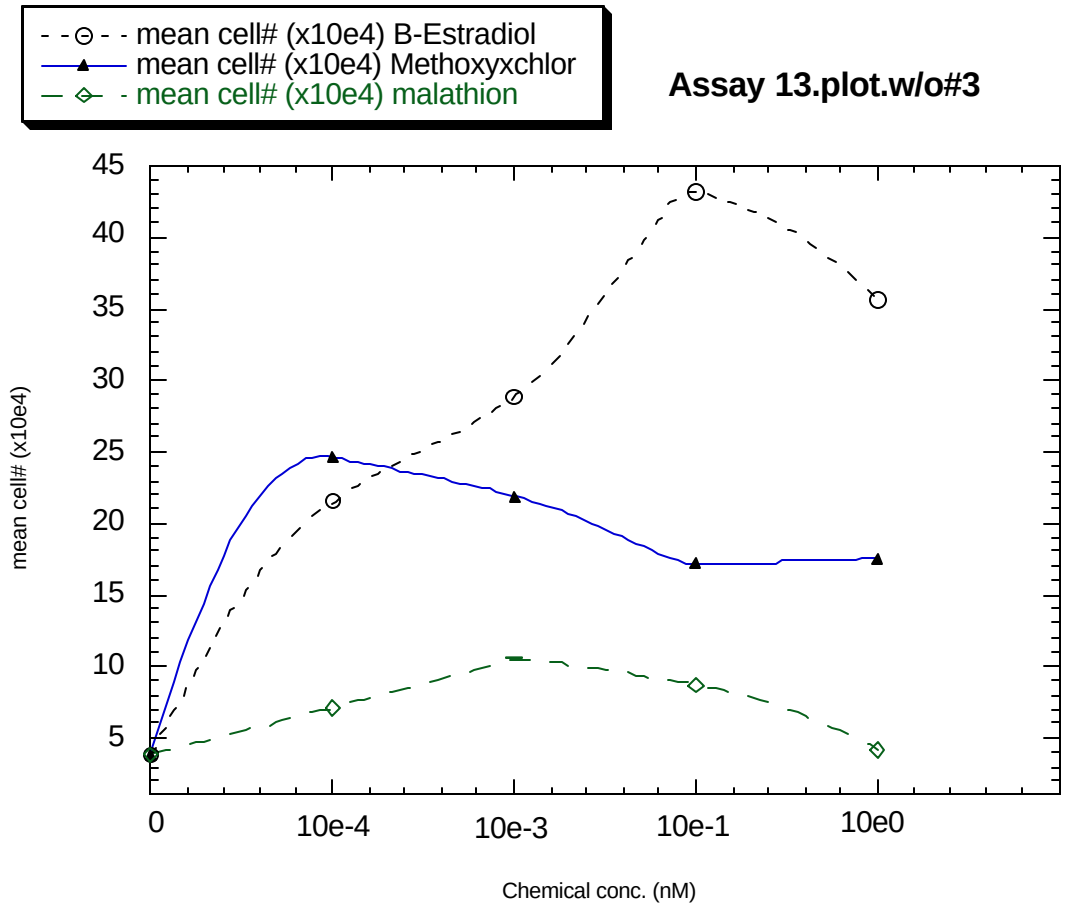

Fig 1. Cell growth in 17 ß-estradiol, compared to cells treated to methoxyclor and malathion.

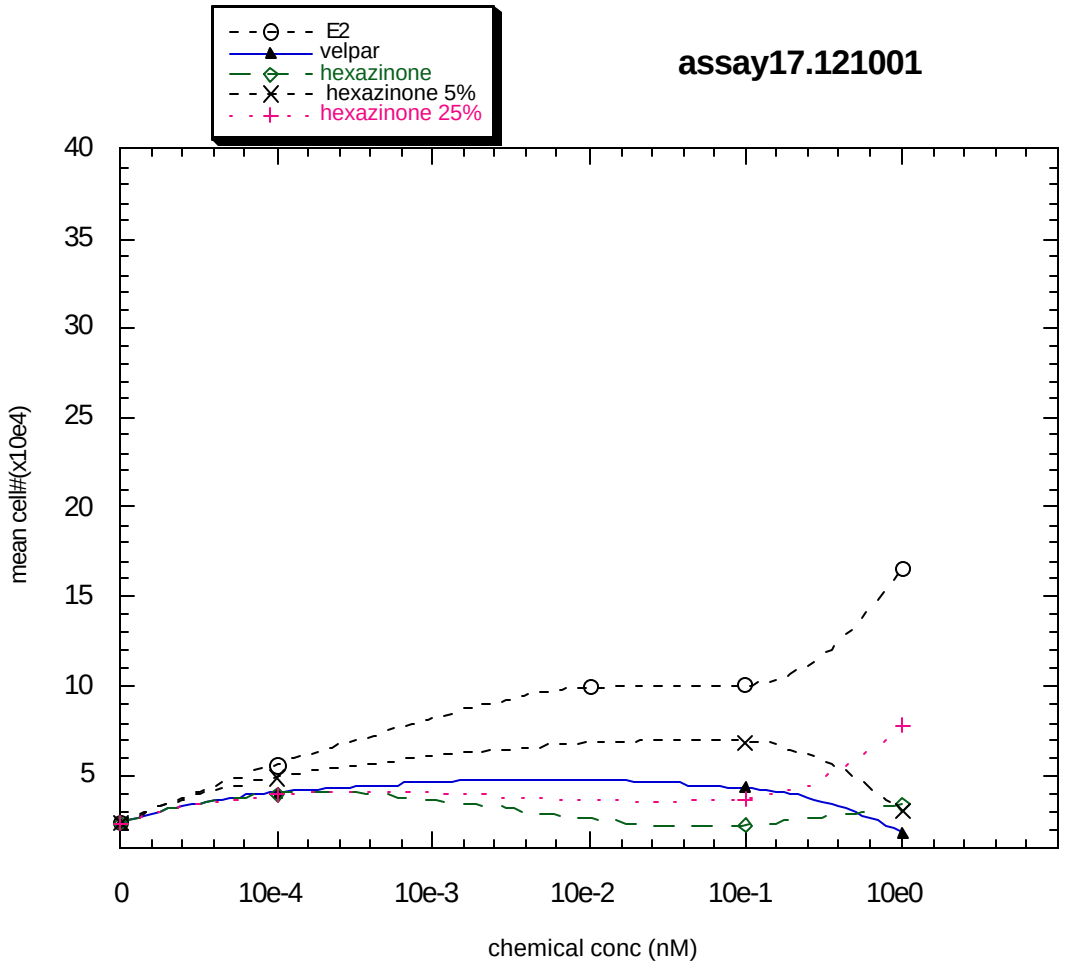

Fig 2. Comparison of cells grown in estradiol (E2) to those exposed to Velpar and its active ingredient hexazinone.

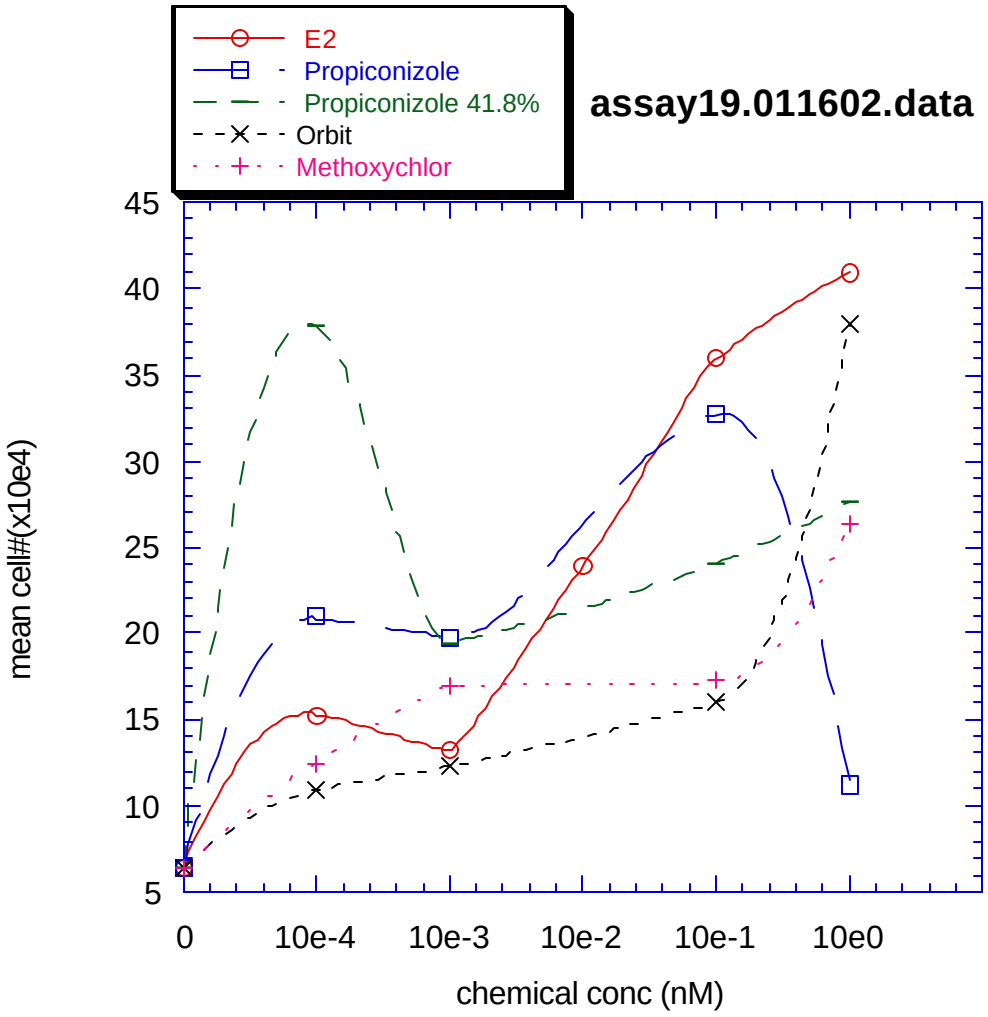

Fig 3. Cell growth in estradiol compared to cells exposed to Orbit, its active ingredient, propriconozol , and methoxychlor.

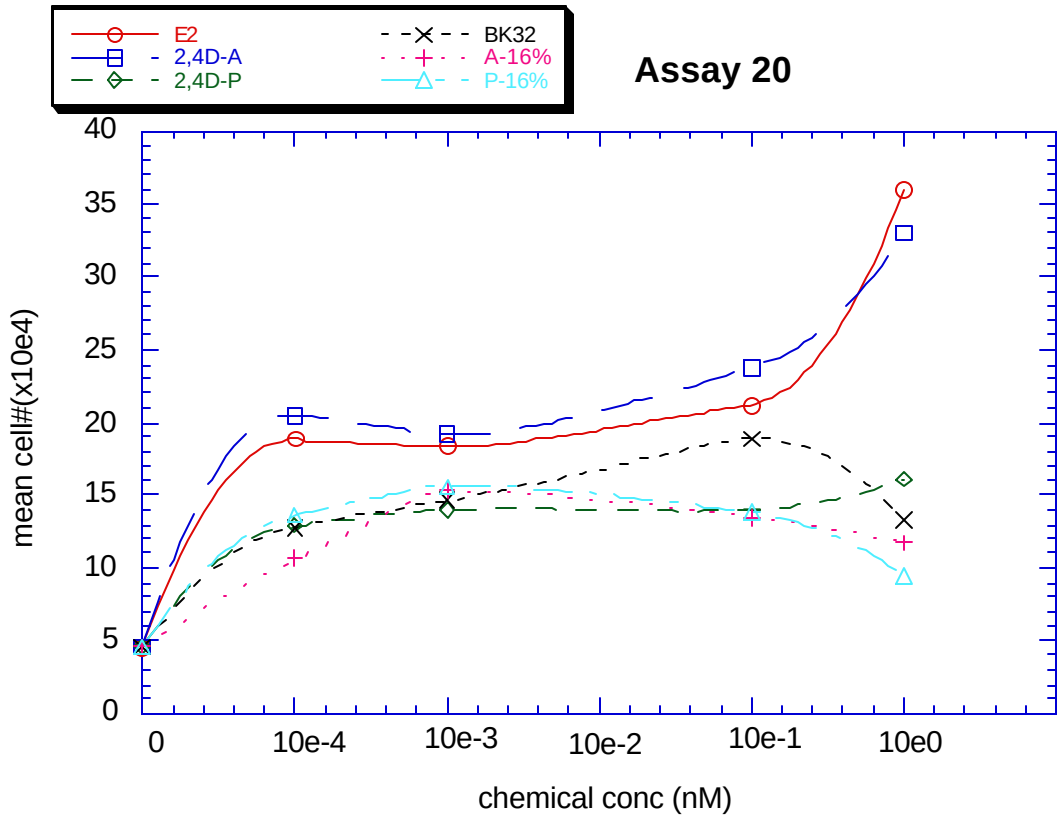

Fig 4. Comparison of MCF-7 cells grown in the presence of estradiol (E2)to those exposed to 2,4 D acetic acid (2,4D-A) and propionic acid (2,4D-P) forms and the formulation Super BK32.